ISSN 0026-2617, Microbiology, 2018, Vol. 87, No. 5, pp. 725–731. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © I.V. Blagodatskikh, O.V. Vyshivannaya, A.V. Alexandrova, E.A. Bezrodnykh, P.V. Zelenikhin, S.N. Kulikov, V.E. Tikhonov, 2018, published in Mikrobiologiya, 2018, Vol. 87, No. 5, pp. 610–617.

EXPERIMENTAL ARTICLES

Antibacterial Activity and Cytotoxicity of Betainated Oligochitosane Derivatives

I. V. Blagodatskikh[a, *], O. V. Vyshivannaya[a], A. V. Alexandrova[a, b], E. A. Bezrodnykh[a], P. V. Zelenikhin[c], S. N. Kulikov[c, d], and V. E. Tikhonov[a]

[a] *Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, 119991 Russia*
[b] *Moscow Technological University, Moscow, 119571 Russia*
[c] *Kazan Scientific Research Institute of Epidemiology and Microbiology, Kazan, 420008 Russia*
[d] *Kazan Federal University, Kazan, Russia*
**e-mail: blago@ineos.ac.ru*

Received March 21, 2018

Abstract—Insolubility of chitosan and oligochitosan in aqueous media above pH 6.2–6.5 and the resulting decreased penetration thorough mucosa restricts significantly their application in biomedical compositions. To create non-cytotoxic chitosan derivatives with a high antibacterial activity in weakly alkaline physiological media, alkali-soluble derivatives of oligochitosan and low-molecular weight chitosan were synthesized by coupling a betaine substituent to the nitrogen atom of glucosamine units. Comparative studies of the pH range of the solubility of these derivatives depending on the chain length and modification degree of oligochitosan with $M_w = 9.7 \pm 1.7$ kDa were carried out. It was found that 12 mol % of betaine substituents was the optimal modification degree ensuring the existence of a single-phase solution of modified oligochitosan ($M_w = 2–10$ kDa) within the entire pH range. Investigation of the antibacterial activity of betaine derivatives against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria revealed their high antibacterial effect in both weakly acidic and weakly alkaline environments. Betaine derivatives were found to be nottoxic towards cow lung epithelial cells (LEC) at concentrations below 2.5 mg/mL. The results obtained seem promising for the application of these derivatives in biomedical compositions.

Keywords: antimicrobial effect, cytotoxicity, chitosan, oligochitosan, quaternization, solubility

DOI: 10.1134/S0026261718050041

The number of antibiotic-resistant bacteria, especially among the agents of clinical infections, is presently increasing dangerously. Research on development of new nontoxic biocidal preparations as alternatives to antibiotics for parenteral application is therefore supported in all developed countries, as well as development of alternative therapeutic approaches based on targeted delivery of the preparations and widespread application of nontoxic biocompatible carriers. Chitosan and its nontoxic derivatives are among the promising carriers of such type. The pronounced mucoadhesive properties and the ability to open tight junctions, i.e., to stimulate absorption of transported compounds via both the transcellular and paracellular pathways are important advantages of chitosan as a basis for transport media. The loss of chitosan solubility at pH above 6.5 and its decreased antimicrobial activity in neutral and alkaline media corresponding to the conditions of mammalian intestine and intercellular space are, however, significant limitations for development of chitosan-based dosage forms.

Introduction of additional ionogenic groups, e.g., of quaternary ammonium groups, which are positively charged within the whole pH range, by chitosan alkylation or by its reaction with a compound containing a quaternary trimethylammonium group is an evident solution to the problem of chitosan solubility (Nud'ga et al., 1973; Muzzarelli et al., 1983; Domard et al., 1986; Loubaki et al., 1989, 1991; Le Dung et al., 1994; Sieval et al., 1998; Zhishen et al., 2001; Hamman and Kotzé, 2001; Snyman et al., 2002; Polnok et al., 2004; Spinelli et al., 2004; Lim and Hudson, 2004; Ignatova et al., 2006). Since chitosan is insoluble in the media used in these reactions and is applied as a dispersion, these processes, in spite of their seeming simplicity, result in nonuniform distribution of trimethylammonium groups along the polymer chain and in partial degradation of the macromolecules.

We have previously proposed (Stepnova et al., 2006, 2007) a soft process for one-stage betainization under homogeneous conditions with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as a binding reagent.